Topical Application of Dieckol Ameliorates Atopic Dermatitis in NC/Nga Mice by Suppressing Thymic Stromal Lymphopoietin Production


TO THE EDITOR
Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease that is characterized by extreme pruritus. AD is considered the first stage of atopic march (Leung et al., 2004). From an immunological aspect, AD is closely linked to the disruption of Th1/Th2 cytokine homeostasis that skews to Th2 immunity (Bieber, 2008). Immunological approaches to the treatment of AD have mainly focused on stimulating Th1 immunity to recover the Th1/Th2 balance (Leung et al., 2004). Thymic stromal lymphopoietin (TSLP) was recently shown to play a critical role in the progress of AD by inducing Th2 immune responses (Soumelis et al., 2002; Zhang et al., 2009). Increased

Abbreviations: AD, atopic dermatitis; BMDC, bone marrow-derived dendritic cell; DC, dendritic cell; DNCB, 2,4-dinitrochlorobenzene; HDM, house dust mite; IKKβ, inhibitor of NF-κB kinase subunit beta; OX40L, OX40 ligand; TSLP, thymic stromal lymphopoietin

Corrected proof published online 5 March 2016

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TSLP production in keratinocytes induces the expression of the OX40 ligand (OX40L) in dendritic cells (DCs), which in turn stimulates the differentiation of naïve CD4+ T cells into Th2 cells to produce Th2 cytokines such as IL-4, IL-5, and IL-13 (Leyva-Castillo, Hener, Michea, et al., 2013). Thus, the TSLP-OX40L axis is considered integral to the induction of a Th2 cell-mediated allergic cascade in AD (Murakami-Satsutani et al., 2014). Indeed, high levels of TSLP expression have been observed in epidermal keratinocytes of AD skin lesions (Leyva-Castillo, Hener, Jiang, et al., 2013a). Therefore, suppressing TSLP may represent a novel therapeutic approach for treating AD by restoring Th1/Th2 balance. Here, we investigated whether dieckol, a phlorotannin from Ecklonia cava (Figure 1a), can suppress TSLP production to reduce Th2 immunity and effectively alleviate AD-like symptoms in an NC/Nga mouse model in vivo. First, we determined whether dieckol affects the production of TSLP in a mouse keratinocyte cell line, KCMH-1, that constitutively produces high amounts of TSLP (Segawa et al., 2014). Dieckol reduced TSLP mRNA and protein levels in KCMH-1 cells (Figure 1b and c). In addition, TSLP expression induced by MC903 in mouse ear skin was reduced by topical application of dieckol (see Supplementary Figure S1 online). NF-κB is an important transcription factor required for TSLP expression. Dieckol significantly reduced NF-κB-dependent luciferase expression in KCMH-1 cells that have high basal activation of NF-κB (Figure 1d). Dieckol blocked IL-1β-induced phosphorylation and degradation of IκBα and NF-κB luciferase activity in mouse keratinocytes (Figure 1e and f, see Supplementary Figure S2a online). Similarly, Joe et al. (2006) showed that dieckol inhibited phorbol myristate acetate-induced NF-κB activation in human dermal fibroblasts (Joe et al., 2006). The results confirm that dieckol suppresses TSLP production by inhibiting NF-κB activation in keratinocytes. In contrast,
IL-1β-induced early growth response protein 1 (EGR-1) expression and phosphorylation of p38, extracellular signal–related kinase, and c-Jun N-terminal kinase were not inhibited by dieckol in Pam212 cells (see Supplementary Figure S2b). To delineate how dieckol interacts with the NF-κB signaling cascade, ligand-independent NF-κB activation was induced by exogenously over-expressing the signaling component in...
293T cells. Dieckol suppressed NF-kB activation induced by inhibitor of NF-kB kinase subunit beta (IKKβ) but not p65 (see Supplementary Figure S3 online), suggesting that dieckol may target IKKβ itself or the complex involving IKKβ but not p65 and its downstream components. We further examined whether the suppression of TSLP production would lead to a decrease in OX40L expression in mouse primary bone marrow-derived DCs (BMDCs). Incubation of BMDCs with conditioned medium from KCMH-1 cells resulted in the induction of OX40L mRNA expression in BMDCs. This induction of OX40L mRNA expression was abolished by dieckol treatment of KC MH-1 cells (Figure 1g), suggesting that inhibition of TSLP production by dieckol in keratinocytes can reduce OX40L expression in DCs.

Next, we investigated whether dieckol can suppress TSLP production and AD-like symptoms in vivo using an NC/Nga mouse AD model. Animal care and the experimental protocols were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Catholic University of Korea (permission no. 2013-020-02). AD-like symptoms were induced in male NC/Nga mice by repeatedly applying a topical allergen—extract of Dermatophagoides farina, a major species of house dust mites (HDM)—and 2,4-dinitrochlorobenzene (DNCB) on the shaved dorsum. Dieckol (1%) was applied topically on NC/Nga mice daily for 4 weeks. Repeated application of HDM/DNCB to NC/Nga mice induced skin dryness, severe erythema, hemorrhage, scarring, dryness, edema, excoriation, and erosion (Figure 2a). Meanwhile, topical application of dieckol greatly improved these AD-like skin symptoms induced by HDM/DNCB as determined by dermatitis score evaluation (Yamamoto et al., 2012). IL-33 functions as a positive regulator of the TSLP-OX40L axis, which initiates and maintains Th2 cell-mediated inflammatory responses (Imai et al., 2013; Murakami-Satsutani et al., 2014).

The differentiation of naïve T cells into Th1 and Th2 cells is regulated by key transcription factors such as T-bet and GATA-3 for Th1 and Th2 cells, respectively. Topical application of dieckol blocked the enhanced expression of GATA-3 in AD-like skin lesions (Figure 2g), whereas the mRNA levels of T-bet were increased (Figure 2h). Topical application of dieckol reduced the levels of Th2 cytokines such as IL-4 and -5 in AD-like skin lesions (Figure 2i and j, see Supplementary Figure S5c and d). The level of IL-13 was also slightly decreased by dieckol (Figure 2k, see Supplementary Figure S5e). In contrast, the level of interferon gamma, a Th1 cytokine, was increased by dieckol (Figure 2l, see Supplementary Figure S5f). These results show that topical treatment of dieckol suppressed Th2-mediated immune responses and promoted Th1 immune responses. Dieckol did not significantly reduce mRNA level of ROR-γt, a hallmark of type 2 innate lymphoid cells (Walker and McKenzie, 2013), increased by HDM/DNCB in the skin (see Supplementary Figure S6 online). Collectively, we show that topical application of dieckol alleviates AD-like symptoms in vivo by suppressing Th2 immunity while stimulating Th1 immunity in skin. The down-regulation of Th2 response by dieckol is mediated through the inhibition of TSLP production in keratinocytes and consequent decrease in OX40L expression in DCs. It needs to be determined in future study whether the increase of Th1 response caused by dieckol is mediated by direct regulation of Th1 cells or by indirect outcome derived from down-regulation of Th2 immunity. These results show that the suppression of TSLP production by phytochemicals, such as dieckol, is an effective strategy to prevent AD symptoms by modulating the balance of Th1/Th2 immune responses.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by a grant from the National Research Foundation of Korea funded by the Korean government (Ministry of Science, ICT and Future Planning: NRF-2014R1A2A1A11051234).

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2015.12.046.

REFERENCES

Phenotypic and Histopathological Tumor Characteristics According to CDKN2A Mutation Status among Affected Members of Melanoma Families

TO THE EDITOR

Highly penetrant mutations in the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene have been identified as major risk factors for melanoma, and they account for between 20% and 50% of familial cases (Goldstein and Tucker, 2001; Kefford et al., 1999). Pathogenic germline mutations at CDKN2A have been associated with malignancies other than melanoma, including breast and pancreatic cancers (Borg et al., 2000; de Snoo et al., 2008; Ghiorko et al., 2012; Goldstein et al., 2006; Potrony et al., 2014), smoking-related cancers of the head and neck, lung cancer, and gastroesophageal carcinomas (Helgadottir et al., 2014; Potjer et al., 2015), as well as central nervous system tumors (Pasmant et al., 2007; Petronzelli et al., 2001). Moreover, there is recent evidence to suggest that familial melanoma cases who are wild type for CDKN2A are not at increased risk for nonmelanoma cancers in contrast to pathogenic mutation carriers (Helgadottir et al., 2014). Distinguishing familial melanoma cases with and without pathogenic CDKN2A mutations may serve to heighten awareness of increased risk for other cancers among carriers in melanoma families. Identifying histopathological and other host features that are associated with inherited pathogenic CDKN2A mutations may aide in this pursuit and also serve to better characterize melanoma heterogeneity and elucidate important pathobiological differences between carriers and noncarriers of pathogenic CDKN2A mutations.

We studied affected members of melanoma families assembled across centers of the GenoMEL consortium and evaluated differences in host and histopathological tumor characteristics between carriers and noncarriers of pathogenic CDKN2A mutations. Written informed consent was obtained for each participant, and individual GenoMEL study center investigations were conducted after approval by their respective institutional review boards. To our knowledge, this study is the largest of its kind and incorporates familial melanoma cases from diverse geographical populations. GenoMEL participants who signed informed consent were asked about their personal melanoma history and to complete a self-administered questionnaire asking about phenotypic characteristics including hair color, eye color, freckling, nevi, burnability (effect of acute sun exposure on skin), and tanning ability (effect of chronic sun exposure on skin). A melanoma family was defined by the presence of three or more cases of verified melanoma, or two cases of verified melanoma in first-degree relatives. Histopathological data were abstracted from pathology or other clinical reports; a centralized pathology review was not performed. Germline DNA was screened for mutations in CDKN2A (exons 1α, 1β, 2, and 3) as previously described (Harland et al., 2008), and pathogenicity was assigned according to Supplementary Table S1 online. Pathogenicity was based on demonstrated (i.e., published) impact on the biological functioning of CDK2A, and putative pathogenicity of specific mutations was based on evidence of cosegregation within melanoma families or bioinformatically inferred impact on CDKN2A function. Participants were classified based on the presence or absence of a pathogenic or putatively pathogenic variant.

We tested whether differences in levels of histopathological or phenotypic factors exist by CDKN2A pathogenic mutation carrier status (α = 0.05).